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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
LANDSMAN, ROBERT S	
ART UNIT	PAPER NUMBER
1647	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)
	09/679,852	BLUMER, KENDALL J.
	Examiner	**Art Unit**
	Robert Landsman	1647

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 July 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-30* is/are pending in the application.

4a) Of the above claim(s) *21-26* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-20 and 27-30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

1. Formal Matters

A. The Request for Reconsideration, filed 7/16/03, has been entered into the record.

B. Claims 1-30 are pending in the application. Claims 21-26 have been withdrawn upon the election of Paper No. 6, filed 2/20/02, as being drawn to a non-elected invention. Therefore, claims 1-20 and 27-30 are the subject of this Office Action.

C. All Statutes under 35 USC not found in this Office Action can be found, cited in full, in a previous Office Action.

2. Claim Rejections - 35 USC § 112, first paragraph - enablement

A. It is brought to Applicants' attention that, in arguing that the claims are not obvious over the prior art, as discussed below, it seems reasonable that the present invention is not enabled for the breadth of claims since, if the requirements of Overton and Blummer need to be met for FRET to be detected, then the claims would need to recite exactly where on the GPCRs the CFP and YFP would need to be attached in order to detect FRET. As stand, the claims recite that CFP and YFP can be attached anywhere on the GPCR and FRET can be detected. Given Applicants' arguments on the strictness of the requirements for FRET detection, Applicants are only enabled for the Examples in the specification. However, a rejection under 35 USC 112, first paragraph will not be made at this time until the issues under 35 USC 103 have been handled.

3. Claim Rejections - 35 USC § 103

A. Claims 1-20 and 27-30 remain rejected under 35 USC 103 for the reasons already of record on page 3 of the Office Action dated 1/13/03. Applicants argue that, even though Miyawaki et al. teach the use of FRET, this procedure was only used to detect an intramolecular and not used to detect a real-time in vivo intermolecular interaction, as recited in the present invention. Applicants argue that detection of FRET requires that three conditions be met (Overton and Blummer, *Methods* 27:324-332, 2002) – (1) the fluorescence donor and acceptor must be separated by less than 100 angstroms, (2) that their dipoles must overlap and (3) that their mobility must be restricted. Applicants argue that Miyawaki et al. satisfy these criteria by covalently attaching CFP and YFP to a single molecule of calmodulin, whose atomic structure had been solved in both the presence and absence of calcium. Therefore, only in the presence of calcium, the attached CFP and YFP domains would be in sufficient proximity to detect FRET. Based on this,

Applicants argue that it would not be likely that FRET could be detected intermolecularly. To further support their position, Applicants further argue that, since the atomic structure of a GPCR was unavailable at the time of the present invention, it would be uncertain that the three criteria set forth by Miyawaki et al. would be met.

These arguments have been considered, but are not deemed persuasive. First, White et al. do teach that the GABA receptors (a GPCRs) form heterodimers by an interaction at their intracellular carboxy-terminal tails (page 679, right column, lines 8-10). In fact, White et al. further provide compelling evidence that a physical association occurs between these receptors at their coiled-coil domains (page 680, sentence bridging left and right columns and the first full paragraph on the right column). Given this data, the artisan would expect that these domains physically interact (i.e. are in contact with each other), which would place these domains at less than 100 angstroms as required by the teachings of Overton and Blummer et al. Similarly, since each member of the GABA dimer physically interacts with the other, it would be expected that this physical interaction would restrict the movement of the coiled-coil domains and would, therefore, restrict the mobility of the attached donor and acceptor molecules. Due to this, the donor and acceptor molecules would be placed in an orientation such that their dipoles overlap. Therefore, regardless of the availability of the atomic structure of a GPCR, which was not even known at the time of the present invention and was only recently confirmed, as argued by Applicants, it would have been obvious for the artisan to use FRET to obtain real-time in vivo intermolecular interactions. Given the teachings of White et al. who show that GABA receptors physically associate at their coiled-coil domains, the artisan would be motivated to attach CFP to the coiled-coil domain of one GABA receptor and a YFP to the coiled-coil domain of the other GABA receptor with the reasonable expectation that these molecules would successfully interact in order to detect FRET.

Applicants further argue that, even if these three criteria of Overton and Blummer have been met, detection of an efficient FRET signal requires that the two proteins of interest interact stably and for much of their lifetimes. This argument has been considered, but is not deemed persuasive. First, the phrase "much of their lifetime" is indefinite and open to interpretation. Respectfully, if Applicants desire to use this argument, then a definition of this phrase, along with supporting literature stating that FRET can only occur upon a specified time of protein interaction, is requested. As stands, Applicants have only cited the criteria of Overton and Blummer et al. and the GABA molecules of White et al. meet these limitations. Therefore, it would be expected that if the donor and acceptor molecules attached to these GABA receptors were in close association for any given time, then FRET would be detected. In fact, Figure 4c of

White et al. demonstrates that the transfection of oocytes with GABA R1a and R1b receptors produce a large inward current for approximately 3 minutes, implying that the GABA receptor oligomer interacts stably for an extensive period.

Applicants also argue that FRET measurements with membrane proteins pose unique problems due to the possibility that the proteins could collide non-specifically with other membrane proteins and can form complexes with unknown lifetimes. Therefore, prior to Applicants' invention, and in the absence of proper controls, it was not known whether FRET could occur as a result of specific and stable interactions between proteins in the membrane. Applicants argue that Hebert et al. did not address these issues. Therefore, Hebert's mere suggestion to use FRET, in the absence of data, does not provide the artisan with a reasonable expectation of success for the real-time in vivo detection of oligomerization using FRET. These arguments have been considered, but are also not deemed persuasive. First, the skill in the art is high, as many researchers have advanced degrees, including research-based Ph.D.'s. Therefore, it would be expected that, in order to obtain reliable data, experiments would only be performed in the presence of proper controls, as this is the cornerstone of any art-accepted experiment. Without proper controls, peer-reviewed work would not be published in respected scientific journals. Therefore, given the fact that proper experiments are only done in the presence of the proper controls, there would be a reasonable expectation of success for the real-time in vivo detection of FRET oligomerization using FRET. Furthermore, White et al. teach a very convincing proper control (Figure 4c) in which transfection of individual GABA receptors alone do not produce the desired effect on inward current as does the heterodimer. These controls can be extrapolated, to the detection of FRET, for example, by tagging one GABA or other GPCR receptor with either CFP or YFP (as a fusion protein or recombinantly via splicing the DNA of both the GPCR and fluorescence molecule) and transfecting a cell with this protein or DNA. If non-specific interactions were to occur to produce FRET, they would be seen in the absence of the second member of the heterodimer. In other words, if FRET were to occur only in the presence of the heterodimer, then no FRET should be detected when only one member of the heterodimer is present.

Finally, Applicants argue that White et al. do not provide evidence that GPCRs are oligomeric in vivo (i.e. associate in native cell membranes) and that their biochemical evidence lacks controls and that detergents may allow for the formation of artifactual aggregates. Therefore, since White et al. did not determine if GABA receptors oligomerize in cell membranes, then the co-immunoprecipitation data may be irrelevant, as discussed by Angers et al. For this reason, the co-immunoprecipitation data of White et al. reveal that biochemical interactions can only occur in virto, which may not be biologically relevant.

Therefore, Angers et al. teach that a direct demonstration of this oligomerization is required in living cells. This, according to Applicants, has first been done in the present invention.

Again, these arguments have been considered, but are not deemed persuasive. Given the combination of results taught by White et al. regarding Figure 4c, whose properly controlled experiment provides evidence of GABA (i.e. GPCR) oligomerization real-time in vivo, along with the evidence that these receptors likely interact via their coiled-coil domains, in part by the demonstration that the physical interaction between GABA receptors is C-terminal-specific, as these receptors do not form homodimers (Figure 2a), the artisan would have been motivated to use FRET to detect GPCR oligomerization real-time in vivo. Though Angers et al. state that co-immunoprecipitation may, itself, be irrelevant, neither Angers et al., nor Applicants address the actual functional evidence for oligomerization as seen in Figure 4c of White et al. which, again, provides evidence for real-time in vivo FRET measurements. Even, *arguendo*, White et al. did not show direct oligomerization, the experiment of Figure 4c, which had proper controls, in light of the information taught by White et al. regarding the coiled coil interactions, would have provided motivation to the artisan to use the teachings of White et al., in conjunction with the teachings of Miyawaki et al. and Hebert et al. to practice the present invention.

Finally, Applicants summarize their arguments by stating that it would not have been obvious for one of ordinary skill in the art to combine Hebert et al.'s suggestion with the teachings of Miyawaki et al, directed to intramolecular interactions, and the inconclusive teachings of White et al. to arrive at the present invention. Applicants argue that the present invention was the first study to definitively demonstrate GPCR oligomerization in intact cells and Applicants' invention now makes it possible to further understand GPCR oligomerization.

In response, the Examiner summarizes the response to Applicants' arguments. According to Angers et al., some experiments, such as those performed by White et al. do not conclusively demonstrate that GPCRs form oligomers in vivo. However, the Examiner states that this conclusion is likely and was made based on findings that GABA receptors not only form heterodimers in vivo, as seen in Figure 4c, but that this physical interaction is C-terminal-specific, as these receptors do not form homodimers (Figure 2a). Therefore, it is likely, in evidence to the contrary, that GABA (GPCR) receptors for oligomers due to an interaction at their tails, probably at their coiled-coil domains. Therefore, due to the physical interaction of GABA receptors, White et al. meet the three criteria set forth by Overton and Blummer regarding the conditions required to detect FRET. In addition, Miyawaki et al. have taught the use of producing a fusion protein comprising a receptor, CYP and FYP for use in FRET detection. Therefore, the techniques to produce labeled proteins with fluorescent compounds by recombinant

techniques, as required by the present invention, were well-known and highly successful at the time of the present invention. Finally, Hebert et al. teach that the use of real-time measurements such as FRET in GPCRs is a logical step in further understanding GPCR interactions.

Therefore, given that White et al. provide evidence that GPCRs physically interact to form heterodimers, it would have been obvious to one of ordinary skill in the art at the time of the present invention to have labeled the GPCRs of White et al. by using the teachings of Miyawaki et al., who teach recombinant techniques for labeling proteins with fluorescent compounds for FRET detection. The artisan would have been motivated to have combined these teachings in view of Hebert et al., who clearly state that the use of real-time measurements such as FRET in GPCRs is a logical step in further understanding GPCR interactions. Therefore, given the teachings of White et al., who demonstrate that GPCRs form oligomers in vivo (Figure 4a), there would have been a reasonable expectation of success in combining the teachings of White et al., Miyawaki et al. and Hebert et al. to practice the present invention since the data obtained by White et al, which meet the criteria as required by Overton and Blummer, make it more likely than not that the detection of FRET would occur in GPCRs real-time in vivo. It is believed that all pertinent arguments have been addressed.

B. Claims 5 and 10 remain rejected and claim 15 is also rejected under 35 USC 103 for the reasons already of record on page 4 of the Office Action dated 1/13/03. The arguments regarding White, Miyawaki and Hebert are addressed above. Applicants argue that Gama do not provide motivation to use truncated receptors since there is no indication that the interaction between GPCRs can be measured, as argued above. However, the claims only recite that the receptors are truncated, not that they are required for FRET. Given the arguments aboce and the teachings of Gama regarding producing truncated receptors there is a high likelihood of success to measure FRET in truncated receptors. It appears that the C-terminal tails interact in the heterodimer. Therefore, if a truncation was produced at, for example, the first 2 residues in the N-terminus, it would not be expected that this would affect oligomerization.

4. Conclusion

A. No claim is allowable.

Advisory information

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Robert Landsman whose telephone number is (703) 306-3407. The examiner can normally be reached on Monday - Friday from 8:00 AM to 5:00 PM (Eastern time) and alternate Fridays from 8:00 AM to 5:00 PM (Eastern time).

If attempts to reach the examiner by telephone are unsuccessful, the Examiner's supervisor, Gary Kunz, can be reached on (703) 308-4623.

Official papers filed by fax should be directed to (703) 308-4242. Fax draft or informal communications with the examiner should be directed to (703) 308-0294.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Robert Landsman, Ph.D.
Patent Examiner
Group 1600
September 29, 2003

ROBERT LANDSMAN
PATENT EXAMINER